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Fermentability of corn–lablab bean mixtures from different planting densities

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ABSTRACT

This study was conducted to determine silage fiber characteristics and fermentation profiles of corn (*Zea mays* L.) grown in mixture with lablab bean [*Lablab purpureus* (L.) Sweet] at different planting densities. The experiment was conducted in two environments in 2005. 'Rongai' lablab bean and corn were intercropped at eight different planting densities; 20/80, 40/0, 40/40, 40/80, 60/0, 60/40, 60/80, and 80/80 thousand corn/lablab bean plants/ha. Corn in monoculture or in mixture with bean was harvested between 1/2 and 3/4 milk line. Two 1-L glass jar mini-silos per treatment per replicate plot were filled at a density of 500 g/L, eight jars per treatment per location, and stored for 40 days at room temperature (~22°C). At the time of ensiling a 500-g fresh sub-sample was also taken for dry matter (DM) and initial characterization of the corn and corn–bean mixtures. Each silo was analyzed for fiber characteristics, pH, and fermentation products. Silage crude protein (CP) concentration was on average 17.5% (86.4 g/kg DM) greater in the mixture than monoculture corn (73.5 g/kg DM). The *in vitro* true digestible DM (IVTDDM) concentrations in the mixtures were on average 4.6% less (796 g/kg DM) than that of monoculture corn (834 g/kg DM), but neutral detergent fiber digestibility was not different between monoculture corn and corn–lablab mixtures ($P>0.05$). Lactate concentration was 21.2% higher in the corn–lablab bean mixtures (60.5 g/kg DM) than monoculture corn

Abbreviations: DM, dry matter; IVTDDM, *in vitro* true digestible dry matter; CP, crude protein; TN, total nitrogen; ADF, acid detergent fiber; aNDF, amylase-treated neutral detergent fiber; NDFD, neutral detergent fiber digestibility.

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(49.9 g/kg DM). The contribution of the lablab bean to the mixture increased as corn planting density decreased. Thus the maximum benefits to increased CP occurred in the mixtures with the lowest corn planting densities, but those mixtures also had the greatest reductions in IVTDDM. However all treatments fermented well in spite of significant ($P < 0.05$) differences in pH and fermentation products.

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1. Introduction

Silage making is one of the principal means of forage preservation around the world (Wilkinson et al., 2003). The principle of silage fermentation is the preservation of nutritive value of forages through the anaerobic fermentation of plant water-soluble carbohydrates by lactic acid-producing bacteria (Pahlow et al., 2003). Corn is the main crop for silage in the United States, but one of the major nutrient limitations of corn silage is its low-crude protein (CP) concentration. Corn silage has a high-net energy of lactation that ranges from 1.35 to 1.45 Mcal/kg dry matter (DM) (NRC, 2001) and digestibility that ranges from 659 to 680 g/kg DM, but CP concentration is low, from 70 to 80 g/kg DM (Darby and Lauer, 2002). The inclusion of additives, such as urea, during the ensiling process increases CP concentration, but urea extends silage fermentation, potentially increasing dry matter losses (Kung et al., 2003).

One viable option to increase CP concentration in corn silage is intercropping corn with legumes in the field. There is evidence that intercropping winter wheat (Contreras-Govea et al., 2006) or corn (Titterton and Maasdorp, 1997; Contreras et al., 2008) with legumes has increased CP concentration in the silage. Additionally, DM, hemicellulose, and lactate concentrations also increased in the mixture (Eichelberger et al., 1997; Dawo et al., 2007). Anil et al. (2000) reported that intercropping corn with three different legumes at 70, 160, and 270 g/kg total DM increased crude protein, neutral detergent fiber, and lactate concentration in the mixture as the proportion of legume increased, without affecting significantly pH and ammonia concentration.

It is well documented that legumes have higher buffering capacities than grasses, which tend to extend fermentation, slow the drop of pH, and increase proteolysis (McDonald et al., 1991; Albrecht and Muck, 1991). Moreover, it is also well documented that not all legumes ferment equally when in monoculture (Mustafa and Seguin, 2003; Owens et al., 1999; Albrecht and Beauchemin, 2003) or in mixture (Dawo et al., 2007). Contreras et al. (2008) conducted a study to assess the fermentation characteristics of corn in monoculture or mixture with one of three climbing beans. They found that CP concentration increased on average 12.6%, with lablab bean as the best combination with corn. However, at this moment it is not known how lablab bean density could affect silage fermentation and quality under different corn densities. Therefore, the objective of this study was to determine the effect of lablab bean and corn planting densities on the crude protein concentration, fiber digestibility and fermentation profiles of the resulting mixture silages.

2. Materials and methods

2.1. Field management

This study was conducted during summer 2005 at the University of Wisconsin Arlington (43°18' latitude N, 89°21' longitude W) and Lancaster (42°50' latitude N, 90°47' longitude W) Agricultural Research Stations. Crops at Arlington were grown on Plano silt loam (fine-silty, mixed, mesic Typic Argiudoll), and at Lancaster on Rozetta silt loam (fine-silty, mixed, superactive, mesic Typic Hapludalfs). Soil fertility levels at both locations were maintained at optimal levels for corn silage production (Kelling et al., 1991). Corn was sown alone or in mixture with 'Rongai' lablab bean. Corn-bean planting density combinations were 20/80, 40/0, 40/40, 40/80, 60/0, 60/40, 60/80, and 80/80 thousand plants/ha. Corn was sown in early May, and beans were sown in rows 15 cm beside the corn rows 2 weeks later. Corn and corn-lablab bean mixtures were harvested on 7 and 15 September 2005 for

Lancaster and Arlington, respectively, at a corn maturity stage of 1/2 and 3/4 milk line, respectively. The experimental design was a factorial experiment with four replicate plots per treatment at each location.

2.2. Ensiling process

Monoculture corn and corn–lablab mixtures were chopped with a three-point mounted one-row chopper. Two 1-L glass jars per replicate plot were filled in the field at a density of 500 g/L, eight jars per treatment at a location, and stored for 40 days at room temperature ($\sim 22^{\circ}\text{C}$) without inoculation. At the time of ensiling a 500-g fresh sub-sample was taken for dry matter and initial characterization of corn and corn–lablab bean mixtures, including the proportion of bean in each mixture. After 40 days, silos were frozen to -20°C to stop fermentation and remained frozen until analysis.

2.3. Silage analysis

Silages were analyzed for pH by placing a 20-g sample in a blender jar, diluting with deionized distilled water to 200 g, and blending for 30 s in a high-speed blender. The diluted sample was filtered through four layers of cheesecloth, and pH was measured with a pH meter immediately. A 20 mL aliquot was centrifuged at $25,000 \times g$ for 20 min at 4°C , and supernatant was decanted into 20-mL scintillation vials and stored at -20°C for later analysis of organic acids. Organic acids (succinate, lactate, acetate, propionate, and butyrate) and ethanol were determined using high-performance liquid chromatography (Muck and Dickerson, 1988). The HPLC system consisted of a Shimadzu system controller (SCL-10A), pump (LC-10AT), and refractive index detector (RID-6A) (Shimadzu Corp., Kyoto, Japan) with a Bio-Rad Aminex HPX-87H column (Bio-Rad Lab, Hercules, CA) heated to 42°C . Flow rate was 0.6 mL/min with a mobile phase of 0.015N H_2SO_4 and 0.25 mM ethylene diamine tetraacetic acid.

At the time of silo opening, duplicate samples (50 g) were taken for moisture determination by freeze-drying. After moisture determination, the duplicate freeze-dried samples were ground to 1 mm particle size and analyzed for total nitrogen (TN) by a Leco FP-2000A nitrogen analyzer (Leco Corp., St. Joseph, MI), sequential heat stable amylase-treated neutral detergent fiber (aNDF)-acid detergent fiber (ADF) with sodium sulfite batch procedures as outlined by ANKOM Technology Corp. (Fairport, NY), and *in vitro* true digestible dry matter (IVTDDM) determined using the Daisy II system (ANKOM Technology Corp., Fairport, NY). The neutral detergent fiber digestibility (NDFD) was calculated following the procedure of Mertens (2006). Ammonia and free amino acids (Broderick et al., 2004) were determined using flow-injection (Lachat Quik-Chem 8000 FIA; Lachat Instruments, Milwaukee, WI).

2.4. Statistical analysis

Data analyses were conducted using the MIXED Procedure of SAS (SAS, 2001). Corn–lablab bean mixtures were considered fixed effects while location and block were considered random effects. When treatment effect was significant, means were separated using LSMEANS (SAS Institute, 2001) with the PDIF option ($P < 0.05$). Correlation analysis was conducted using the CORR Procedure of SAS (SAS, Institute, 2001).

3. Results

3.1. Lablab bean proportion in the mixture

There was a negative correlation ($r = -0.87$) between lablab bean proportion in the mixture, on dry matter basis, and corn plant density. As corn planting density increased from 20 to 80 thousand plants/ha, lablab bean proportion in the mixture decreased independently of bean planting density. The correlation analysis between amount of bean in the mixture and nutritive value parameters was significant for the majority of parameters (Table 1). Crude protein, aNDF, ADF, ammonia, lactate, and acetate

Table 1Pearson correlations (*r*) between lablab bean concentration in the mixture and silage fermentation characteristics^a

Parameter	<i>r</i>	P-value
DM	−0.901	0.0001
CP	0.722	0.0016
aNDF	0.726	0.0014
ADF	0.716	0.0018
Hemicellulose ^b	0.243	0.3638
IVTDDM	−0.714	0.0019
NDFD	−0.209	0.4373
pH	0.325	0.2193
Lactate	0.876	0.0001
Acetate	0.790	0.0003
Ethanol	−0.163	0.5475
Ammonia-N	0.853	0.0001
Free amino acids	0.098	0.7104

^a DM, dry matter; CP, crude protein; aNDF, amylase-treated neutral detergent fiber; ADF, acid detergent fiber; IVTDDM, *in vitro* true digestible dry matter; NDFD, neutral detergent fiber digestibility.

^b aNDF-ADF.

concentration were positively correlated with lablab bean concentration in the mixture ($P<0.05$), while DM concentration and IVTDDM were negatively correlated with bean concentration ($P<0.05$). Hemicellulose, NDFD, free amino acids, pH, and ethanol concentration were not significantly correlated with bean proportion ($P>0.05$).

3.2. Fiber fermentation characteristics

The DM concentration of monoculture corn, average of 40/0 and 60/0 treatments (393 g/kg) was similar to the mixture with higher corn and lablab bean density (80/80) (386 g/kg), but greater than the other mixtures ($P<0.05$, Table 2). Averaging over monoculture corn and mixtures, corn–lablab bean mixtures had 14.7% lower DM concentration (335 g/kg) than monoculture corn. The lowest DM concentration was in the mixture with the lowest corn–highest lablab bean densities, 20/80 (276 g/kg), indicating the effect of higher bean proportion in the mixture. The regression analysis between DM concentration and amount of bean (Table 3) indicated that for each 100 g of lablab bean per kg of mixture, DM concentration decreased 32.7 g/kg.

Averaging over mixtures, crude protein concentration was 17.5% (86.4 g/kg DM) greater than monoculture corn (73.5 g/kg DM). The biggest increases in CP concentration were observed with mixtures 20/80 (108 g/kg DM) and 40/40 (90 g/kg DM), which agree with the greater lablab bean concentrations in those mixtures. The regression analysis indicated that for each 100 g of bean per kg of mixture (Table 3), protein concentration increased 10.4 g/kg DM.

The aNDF and ADF concentrations were different between monoculture corn and corn–lablab bean mixtures ($P<0.05$, Table 2). Both aNDF and ADF concentrations increased as the amount of lablab bean increased in the mixture. Averaging monoculture corn treatments and mixtures, aNDF concentration in the mixtures (330 g/kg DM) was 21.9% greater than that in monoculture corn (271 g/kg DM); whereas ADF concentration in the mixtures (199 g/kg DM) was 37.4% greater than in monoculture corn (145 g/kg DM). Among mixtures, corn–lablab bean mixture 20/80 had the greatest aNDF and ADF concentrations (349 and 204 g/kg DM, respectively), whereas mixture 80/80 had the lowest aNDF concentration (299 g/kg DM) and mixture 60/40 the lowest ADF concentration (189 g/kg DM). The linear regressions between aNDF and ADF and lablab bean concentration (Table 3) indicated that for each 100 g increment of lablab bean per kg of mixture, aNDF and ADF increased 25 and 21 g/kg DM, respectively.

Opposite to aNDF concentration, IVTDDM decreased as the amount of lablab bean increased in the mixture ($P<0.05$, Table 2). Corn without bean (40/0 and 60/0) (834 g/kg DM) or with the lowest bean proportion (80/80) (811 g/kg DM) had similar ($P>0.05$) IVTDDM concentrations but 5.2% greater IVTDDM than the average of the other corn–lablab bean mixtures (780 g/kg DM). The linear equation

Table 2Fiber characteristics^a of corn and corn–lablab bean mixture silages (pooled values over two environments)

Corn (×1000 plants/ha)	Lablab (×1000 plants/ha)	Bean (g/kg)	DM (g/kg)	CP (g/kg DM)	aNDF (g/kg DM)	ADF (g/kg DM)	HC ^b (g/kg DM)	IVTDDM (g/kg DM)	NDFD g/kg aNDF
40	0	0	377	78	281	152	130	827	387
60	0	0	408	69	261	137	123	840	389
80	80	124	386	73	299	193	107	811	368
60	80	146	357	71	327	190	137	789	357
60	40	148	357	76	326	189	137	789	360
40	40	213	323	90	339	208	132	776	347
40	80	245	311	87	342	211	131	786	379
20	80	380	276	108	349	204	145	762	324
S.E.M.			11.1	5.5	15.7	12.3	9.2	14.5	19.0

^a DM, dry matter; CP, crude protein; aNDF, amylase-treated neutral detergent fiber; ADF, acid detergent fiber; HC, hemicellulose; IVTDDM, *in vitro* true digestible dry matter; NDFD, neutral detergent fiber digestibility.

^b aNDF-ADF.

Table 3

Relationship between different parameters^a (g/kg dry matter except as noted) and lablab bean concentration (g/kg mixture on a dry matter basis)

Parameter	Regression equation	R ²
Dry matter (g/kg)	$Y = -0.327x + 400.57$	0.81
Crude protein	$Y = 0.104x + 65.29$	0.54
aNDF	$Y = 0.251x + 276.30$	0.53
ADF	$Y = 0.210x + 152.48$	0.51
IVTDDM	$Y = -0.228x + 833.27$	0.51
Ammonia-N (g/kg total N)	$Y = 0.037x + 29.53$	0.73
Lactate	$Y = 0.064x + 47.74$	0.77
Acetate	$Y = 0.027x + 9.42$	0.63

^a aNDF, amylase-treated neutral detergent fiber; ADF, acid detergent fiber; IVTDDM, *in vitro* true digestible dry matter.

between IVTDDM and lablab bean concentration (Table 3) indicated that for every 100 g of lablab bean per kg mixture, IVTDDM diminished 23 g/kg DM.

Hemicellulose concentration and NDFD were not different between monoculture corn and lablab bean mixtures ($P > 0.05$, Table 2). Though differences were not significant ($P > 0.05$), there was a trend toward greater hemicellulose concentration and lower NDFD in the mixtures (131 and 356 g/kg DM) than monoculture corn (126 and 388 g/kg DM).

Free amino acids concentrations were not different between monoculture corn and mixtures ($P > 0.05$), but they were different in ammonia concentration ($P < 0.05$, Table 4). On average, the mixtures had 24% greater ammonia formation than monoculture corn. The regression equation showed that ammonia concentration increased 3.7 g/kg TN for each 100 g of lablab bean per kg of mixture (Table 3).

3.3. Products of fermentation

The pH, lactate, and acetate concentrations were different among treatments ($P < 0.05$), but ethanol was not ($P > 0.05$) (Table 4). Butyric acid was not detected in any treatment. On average, the pH of monoculture corn silage was 0.12 units lower than that of the mixtures. The correlation between lablab bean concentration and pH was not significant ($P = 0.21$). Among mixtures, the lowest pH was with mixture 40/80 (pH 3.89), while the highest pH was with mixture 80/80 (pH 4.07).

Lactate concentration was 21.2% lower on average in monoculture corn (49.9 g/kg DM) than in the corn–lablab mixtures (60.5 g/kg DM) (Table 4). Among mixtures, as the proportion of lablab bean increased, lactate concentration increased from 52.0 to 72.0 g/kg DM. The regression analysis between bean in mixture and lactate concentration (Table 3) indicated that for each 100 g increase in lablab bean per kg of mixture, lactate concentration increased 6.4 g/kg DM.

A similar trend to lactate was observed with acetate concentration (Table 4). Acetate concentration was 37.4% lower on average in monoculture corn (10.7 g/kg DM) than in the corn–lablab bean mixtures (14.7 g/kg DM). Among mixtures, acetate concentration increased 71.8% as the concentration of bean increased from 124 g/kg DM (acetate = 11.7 g/kg DM) to 380 g/kg DM (acetate = 20.1 g/kg DM). The regression analysis indicated that acetate concentration increased 2.7 g/kg DM for each 100 g/kg DM of bean per kg of mixture (Table 3).

4. Discussion

Previous studies reported that crude protein concentration in corn for silage can be increased when intercropping corn with different climbing beans (Armstrong et al., 2008) or with lablab bean (Armstrong and Albrecht, 2008) without significantly affecting dry matter yield. We also reported that fermentation characteristics of corn silage in mixture with climbing beans were different than corn in monoculture, depending on the contribution of bean in the mixture (Contreras-Govea et al., 2008). In this study the objective was to determine how the density of lablab bean in mixture with corn affects crude protein concentration, fiber digestibility, and fermentation profiles of silage.

Table 4
Fermentation characteristics of monoculture corn and corn–lablab bean mixture silages (pooled values over two environments)

Corn (×1000 plants/ha)	Lablab (×1000 plants/ha)	Bean (g/kg)	FAA ^a (g/kg TN)	Ammonia (g/kg TN)	pH	Lactate (g/kg DM)	Acetate (g/kg DM)	Ethanol (g/kg DM)
40	0	0	186	30	3.83	52.6	11.0	5.7
60	0	0	190	30	3.86	47.2	10.3	6.8
80	80	124	189	34	4.07	52.0	11.7	5.6
60	80	146	186	34	3.93	53.8	12.3	5.3
60	40	148	184	36	3.94	56.8	13.2	5.9
40	40	213	181	39	3.99	63.1	16.1	5.0
40	80	245	179	36	3.89	65.2	14.7	5.7
20	80	380	190	44	3.95	72.0	20.1	5.9
S.E.M.			6.9	1.8	0.038	2.57	1.29	0.77

^a FAA, free amino acids; TN, total N; DM, dry matter.

4.1. Crude protein concentration and fiber digestibility

As expected, CP concentration in the silage increased as the concentration of lablab bean increased. However, corn planting density was the principal factor that determined the amount of bean in the mixtures and subsequently the increase in CP concentration; doubling bean planting density (40- or 80 thousand bean plants/ha) appeared to have no significant effect on the amount of bean in the mixtures and thus on CP concentration (Table 2). Therefore, the greatest increase in CP concentration (47%) relative to monoculture corn was with corn–bean density of 20/80 thousand plants/ha. This finding agrees with Titterton and Maasdorp (1997) who ensiled soybean–corn mixtures from 0 to 800 g soybean/kg mixture and found a linear relationship between soybean proportion and CP concentration. Similarly, Dawo et al. (2007), who conducted a study intercropping corn with bean (*Phaseolus vulgaris*), also reported that decreasing corn density increased CP concentration in the silage mixture. In another study, Armstrong and Albrecht (2008), who intercropped corn with lablab bean, reported a positive and linear correlation ($r = 0.62$) between CP concentration and proportion of bean, which agrees with this finding (Table 3). Therefore, it was clear that mixing corn with lablab bean increased CP concentration in the silage, but the impact or contribution of lablab bean in the mixture depended on corn planting density.

The cell wall constituents aNDF (hemicellulose, cellulose, and lignin) and ADF (cellulose and lignin) are negatively correlated with IVTDDM (Theander and Westerlund, 1993). High-quality forages have low concentrations of both aNDF and ADF and high digestibility (Paterson et al., 1994). Moreover, grasses, like corn, and legumes, like lablab bean, differ in aNDF and ADF concentration. Grasses are higher in hemicellulose and lower in lignin than legumes (Hatfield, 1993), which may have an effect on digestibility. In this study, the silage mixtures had increased aNDF and ADF concentrations and decreased IVTDDM as the amount of bean increased in the mixture, compared with monoculture corn (Table 3). Therefore, these results are in agreement with Hatfield (1993), that increasing aNDF and ADF concentrations decrease digestibility. However, the lack of significance in hemicellulose and NDFD between monoculture corn and mixtures could indicate that the beans did not contribute significantly different amounts of hemicellulose than the corn to the mixtures but did increase cellulose and lignin (not measured in this study but implied by the higher ADF concentrations of the mixtures). Titterton and Maasdorp (1997) reported a similar effect when ensiling corn with soybean, and Contreras-Govea et al. (2008) when ensiling corn with different climbing beans.

4.2. Fermentation profiles

Legumes like lablab bean are expected to have higher silage pH, ammonia formation and lactic acid production than grasses as a result of greater buffering capacity (McDonald et al., 1991; Muck et al., 2003). In this study, ammonia formation was greater in the mixtures than monoculture corn, pH tended to be higher in the mixtures than monoculture corn, and lactate and acetate were greater in the mixture than monoculture corn (Table 4). It is likely that there was a surplus of water-soluble carbohydrates (not measured in this study) from the corn even in the 20/80 mixture with the highest proportion of bean and that the higher buffering capacity of the bean extended the fermentation period, resulting in greater lactate and acetate concentrations. These results agree with those of Dawo et al. (2007) and Titterton and Maasdorp (1997) who also reported higher pH, lactic acid and ammonia with an increase in legume concentration when in mixture with corn.

5. Conclusions

Decreasing corn-planting density increased the amount of lablab bean in the mixture when the two crops were intercropped. As the proportion of lablab bean in the mixture increased, silage CP increased and IVTDDM decreased. All mixtures fermented well but with higher silage pH and increased lactate and acetate concentrations than corn silage. At a corn planting density of 40 thousand plants/ha (~50% of normal) and bean density ranging from 40 to 80 thousand plants/ha, silage CP was increased 20% (from 73.5 to 88.5 g CP/kg DM) while IVTDDM was reduced 6% (from 834 to 781 g/kg DM). Additional

research is needed to examine the effects of these intercropped silages on animal performance relative to corn silage.

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